Rapid microbial identification and phenotypic antimicrobial susceptibility testing directly from positive blood cultures: a new platform compared to routine laboratory methods

Rapid microbial identification and phenotypic antimicrobial susceptibility testing directly from positive blood cultures: a new platform compared to routine laboratory methods.

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Abstract

The Accelerate Pheno™ System (APS), a new platform that combines rapid identification (ID) of bacteria and yeasts and phenotypic antimicrobial susceptibility testing (AST) in a single assay, has been evaluated directly from positive blood cultures in comparison to routine laboratory methods. The APS ID results showed an overall sensitivity and specificity of 92.6% and 99.6%, respectively. With regard to AST results, 31 discrepancies (8 single errors and 23 combined errors) were observed, including 13 major errors (3.3%) and 18 minor errors (4.6%) mainly involving *Pseudomonas aeruginosa*. No very major error was observed. The APS ID results were obtained in 1.5 h and the AST results were available in 7 h, on average 34.1 h before routine laboratory methods. This reduction in AST time-to-result represents one of the main advantage of this technology, reducing the time to provide to the physician the microbiological report. ~~optimize a targeted therapy and allowing to improve clinical outcomes~~.

Keywords: rapid identification; phenotypic antimicrobial susceptibility testing; blood culture; antimicrobial therapy

1. Introduction

Rapid identification (ID) and antimicrobial susceptibility testing (AST) of microorganisms from blood specimens are essential to assure appropriate patient management (Retamar et al. 2012; Calderaro et al. 2014; Rhodes et al. 2017). The timely administration of targeted antimicrobial therapy can reduce morbidity and mortality in patients with bloodstream infections as well as prevent the development of antibiotic resistance (Fraser et al. 2006). Automated continuous monitoring blood culture (BC) systems have reduced the delay in detecting the presence of blood-borne bacteria and fungi. However, complete organism ID and AST generally takes 2-5 days after sample collection, necessitating several days of empirical therapy that can be inadequate or suboptimal, risking poor patient outcome (Pardo et al. 2016).

Molecular rapid diagnostic tests, including polymerase chain reaction (PCR), matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry, and peptide nucleic acid fluorescent *in situ* hybridization (PNA-FISH), from positive BCs has improved the conventional laboratory algorithm by reducing the time to organism ID (Calderaro et al. 2014; Timbrook et al. 2017), but they are not able to provide phenotypic AST on the basis of minimal inhibitory concentration (MIC) values.

In our laboratory the microbial ID from positive BCs is detected by a combination of methods, such as FISH (Calderaro et al. 2014) and MALDI-TOF assays, both also used for the detection of genotypic and phenotypic resistance markers, together with culture-based conventional AST methods.

Recently, a new platform, the Accelerate Pheno™ System (APS, Accelerate Diagnostics) that combines ID and AST in a single assay producing an ID within 90 min and a phenotypic AST in approximately 7 h from a positive BC, has become commercially available.

The purpose of this study was to evaluate the performance of the innovative APS for ID and AST directly from BCs in comparison to routine laboratory methods. This is one of the few manuscripts evaluating APS in laboratory practice after its commercial availability, if excluding those reporting its validation.

2. Material and Methods

2.1 Blood cultures

Blood samples sent to the Unit of Clinical Microbiology of the University-Hospital of Parma for routine diagnostic purposes were collected in Bactec Plus aerobic/F medium, Bactec Plus anaerobic/F medium or Bactec Peds Plus/F medium bottles (Becton Dickinson, Baltimore, MD, USA) and incubated in a Bactec FX continuous BC monitoring instrument (Becton Dickinson) until growth was detected. Among the 14,302 BCs belonging to 4566 patients received during the year 2018, 2291 BCs belonging to 1109 patients were positive and a total of 61 selected positive BCs were included in the study for the evaluation of the APS. In consideration of the APS total run time (about 8 h) and of the availability of two APS modules, Eeach day (for a 2-month period) a maximum of two positive BCs were tested by APS. The inclusion criteria were: i) fresh positive BCs, within 8 h of growth detection, according to manufacturer's instructions;, ii) the first positive BC (aerobic or anaerobic) of a patient; , and iii) on the basis of Gram-staining, BC containing Gram-negative rods (GNR), Gram-positive cocci (GPC) in chains, GPC in clusters and yeasts, on the basis of Gram-staining, in such order of priority, in the case of more than two positive BCs. Known-off panel organisms on the basis of Gram-staining, such as Gram-positive rods and Gram-negative cocci, were excluded. A total of 61 selected positive BCs were included in the study.

2.2 Clinically-challenging samples

Ten experimentally seeded samples with well-characterized strains (2 *Enterococcus faecium*, 2 *Escherichia coli*, 2 *Staphylococcus aureus*, 1 *Enterobacter cloacae*, 1 *Enterococcus faecalis*, 1 *Klebsiella pneumoniae*, and 1 *Pseudomonas aeruginosa*, supplied by Accelerate Diagnostics, Tucson, AZ, USA) were used to evaluate the performance of the instrument, according to the following

protocol. For each strain, a suspension of 0.5-0.63 McFarland in saline solution was prepared and subsequently diluted 1:100. An aliquot of 0.5 ml was transferred into a Bactec Plus aerobic/F bottle added with 9.5 ml of donor blood (supplied by Accelerate Diagnostics~~, Tucson, AZ, USA~~) and incubated in the Bactec FX instrument, according to standard procedures, as previously described (Calderaro et al. 2014).

2.3 Routine laboratory methods (RLM) for ID and AST

When a BC signaled positive, an aliquot was submitted to Gram staining and in parallel subcultured onto blood agar, MacConkey agar, Schaedler agar and/or Sabouraud dextrose agar with chloramphenicol (KIMA, Piove di Sacco-PD, Italy) and incubated at 37°C in the appropriate conditions according to bottle type and Gram staining result, as previously described (Calderaro et al. 2014). The microorganism ID was initially performed directly from BCs by *Quick*FISH (AdvanDx, Woburn, MA, USA), according to manufacturer's instructions, using four different probe sets on the basis of Gram staining result: "*Staphylococcus Quick*FISH", distinguishing between *S. aureus* and coagulase-negative staphylococci (CoNS), in the case of samples containing GPC in pairs or clusters, "*Enterococcus Quick*FISH", distinguishing between *E. faecalis* and *Enterococcus* spp., in the case of GPC in chains, "Gram-Negative *Quick*FISH", distinguishing among *E. coli*, *K. pneumoniae* and *P. aeruginosa*, in case of Gram-negative rods, and "*Candida Quick*FISH" for yeasts distinguishing among *Candida albicans*, *Candida parapsilosis* and *Candida glabrata*. In the case of a *Quick*FISH-negative result, an ID was performed directly from BCs by MALDI-TOF using Autoflex Speed- MALDI Biotyper version 3.1 (Bruker Daltonics GmbH, Bremen, Germany, supplied by Becton Dickinson) and VITEK MS version 3 (bioMérieux, Marcy L'Etoile, France) mass spectrometers after purification and protein extraction (Scola and Raoult 2009; Spanu et al. 2012). After sufficient (at least two to three colonies) pure microbial growth was achieved (24–72 h), ID was performed from solid culture by Autoflex Speed- MALDI Biotyper mass spectrometer in the case of GNR and by VITEK MS mass spectrometer in the case of GPC and yeasts, according to the laboratory workflow (Calderaro et al. 2014).

Conventional AST was performed by BD Phoenix system using the Gram-negative NMIC/ID94 Combo Panels (Becton Dickinson) for Gram-negative isolates and by Vitek 2 system using the P619 and P586 cards (bioMérieux) for *Staphylococcus* spp. and *Enterococcus* spp., respectively. For the drug/microorganism combination not included in the automated system panels (such as daptomycin for *Enterococcus* spp., doxycycline for *Enterococcus* spp. and *Staphylococcus* spp. and tobramycin for Gram-negative rods), the gradient diffusion test (Liofilchem, Roseto degli Abruzzi, TE, Italy), performed according to standard procedure was used (Isenberg 2007). For *Enterobacteriaceae* the

ampicillin-sulbactam APS result was compared with the amoxicillin-clavulanate one~~. C~~cefazolin was not considered in the comparison due to the lack of the drug either in the automated AST routinely used or as gradient diffusion test.

For AST discrepancy testing, from pure culture the gradient diffusion test was used, except for colistin tested by broth microdilution (Liofilchem), performed following the manufacturer's instructions, and for cefoxitin resistance screening performed by Kirby-Bauer disk diffusion (Oxoid, Hampshire, UK), according to standard procedures (Isenberg 2007). The AST results were interpreted according to MIC breakpoint criteria of the CLSI (CLSI 2018).

The RLM results were used as the reference comparator for bacterial ID and AST. To determine the ID accuracy, the sensitivity and the specificity were calculated by using the *Quick*FISH ID probe for the APS and the results were compared to those of the RLM: sensitivity = (100 X TP)/(TP + FN); specificity = (100 X TN)/(TN + FP), where TP is the true positive, FN is the false negative, TN is the true negative, and FP is the false positive.

For AST accuracy, essential agreement (EA), categorical agreement (CA), very major error (VME), major error (ME), and minor error (MiE) rates were calculated using APS AST results compared to those of the RLM for each antimicrobial test. EA is the percentage of the total test results within one doubling dilution of the reference result. CA is the percentage of the total test results with the same categorical interpretation as the reference result. VME is a resistant isolate by the RLM that tested susceptible by the APS. ME is a susceptible isolate by the RLM that tested resistant by the APS. MiE is an intermediate result by RLM or APS when the other method gave a susceptible or resistant result.

2.4 Accelerate Pheno™ System (APS)

Each test was performed using Accelerate PhenoTest BC kit (Accelerate Diagnostics) by APS instrument, according to manufacturer's instructions. Briefly, the kit, containing all ready-to-use reagents, was brought to room temperature and removed from its packaging. An aliquot (0.5 ml) of each selected positive BC was inoculated in a sample vial included in the ~~an Accelerate PhenoTest BC~~ kit ~~(Accelerate Diagnostics) and~~ subsequently placed in the reagent cartridge, which was directly transferred in the APS instrument (provided with 2 modules, allowing to process 2 samples/run) and processed within 10 min ~~by APS instrument, according to manufacturer's instructions~~. APS is able to identify Gram-negative bacteria, such as *Acinetobacter baumannii*, *Citrobacter* spp. (*Citrobacter freundii* and *Citrobacter koseri*), *Enterobacter* spp. (*E. cloacae* and *Enterobacter aerogenes*), *E. coli*, *Klebsiella* spp. (*Klebsiella oxytoca* and *K. pneumoniae*), *Proteus* spp. (*Proteus mirabilis* and *Proteus vulgaris*), *P. aeruginosa* and *Serratia marcescens*, Gram-positive cocci, such as *S. aureus*, CoNS (*Staphylococcus capitis*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus*

hominis, *Staphylococcus lugdunensis*, *Staphylococcus warneri*), *E. faecalis*, *E. faecium* and *Streptococcus* spp. (*Streptococcus agalactiae*, *Streptococcus gallolyticus*, *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus pneumoniae*), and yeasts including *Candida albicans* and *Candida glabrata*. APS is able to perform AST from all bacteria except from streptococci.

All BCs were anonymized before being tested with APS and only laboratory information were recorded and used in this study.

2.5 Reference bacterial strains

Five ATCC reference strains (*E. coli* ATCC 25922 and ATCC 35218, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *P. aeruginosa* ATCC 27853) were used for the calibration of the APS: the ATCC panel was performed three times for each instrument before the first use and subsequently every 15 days, according to the manufacturer's instructions.

3. Results

3.1 Identification

The APS ID results obtained on the challenged samples were concordant with regard to the targets included in the assay in all cases (Table 1).

When APS was used on the 61 clinical specimens, a final result was achieved in 53 cases: 50 containing at least one microorganism (47 monomicrobials and 3 polymicrobials, for a total of 53 microorganisms) and 3 negatives. All APS ID results were in agreement, with regard to the targets included in the assay, with those obtained by the RLM except in 6 cases (Table 2). In two cases APS additionally revealed *P. aeruginosa* besides *S. aureus*, in three cases APS missed to recognize one know-on panel microorganism revealed by RLM (1 *P. aeruginosa* and 1 *S. haemolyticus*, both present in combination with another organism, and 1 *S. pneumoniae*) and in the last case the APS identified CoNS instead of *S. aureus* detected by RLM. For the 8 remaining samples out of the 61 examined samples, the ID was not provided, due to a technical failure (power failure preventing) in six cases and to an indeterminate result, due to too few cells for positive ID, in two cases. The APS showed an overall sensitivity of 92.6%, ranging from 80%-100% among GPC to 85.7%-100% among GNR, and a specificity of 99.6%, varying from 97.9%-100% for GPC to 95.7%-100% for GNR, if excluding yeasts due to the limited number of tested specimens (Table 3).

3.2 Antimicrobial susceptibility testing

The AST results by APS were produced for 9 out of the 10 challenged strains with a total of 73 organism-antimicrobial test results (28 Gram-positives and 45 Gram-negatives, Table A.1 and A.2).

All results were in agreement with those expected. In the sample for which no AST result was achieved, an excess of microbial growth was revealed by the instrument.

With regard to clinical samples, the AST results were generated by APS in 41 of the 50 samples for which an ID was achieved (13 Gram-positives and 28 Gram-negatives), for a total of 390 drug/microorganism combinations. There were initially 32 discrepancies all resolved in favor of RLM by discrepancy analysis except one, involving colistin in an *E. coli* strain resolved to agree with APS result. Therefore, in the final analysis, there were 15 samples with 31 discrepancies (8 single errors and 23 combined errors) including 13 MEs (3.3%) and 18 MiEs (4.6%) (Table 4).

The MEs involved ceftazidime, cefepime, colistin, erythromycin, meropenem, and piperacillin-tazobactam whereas the MiEs involved the same drug except colistin with addition of amikacin, aztreonam, ciprofloxacin, and gentamicin.

Four out of the 8 single errors were MEs involving 2 *E. coli* and 1 *A. baumannii* tested against colistin, and 1 *S. aureus* tested against erythromycin, which resulted resistant by RLM for either *Streptococcus salivarius* or *S. mitis* contained in the same sample and for which no AST was performed by APS. Moreover, in this latter sample a ME involving a methicillin-resistant *S. aureus* (MRSA) by APS was observed. The remaining 4 single MiEs involved 2 *Enterobacteriaceae* tested against cefepime and gentamicin, 1 *P. aeruginosa* tested against amikacin and 1 *S. aureus* tested against erythromycin. The remaining 23 errors were revealed in 7 samples with combination of MEs and MiEs in all cases except one containing *E. coli* with 2 MiEs. The associations of MEs (9) and MiEs (12) was observed in 6 samples: 5 containing *P. aeruginosa* alone (accounting overall for 8 MEs and 10 MiEs) and 1 containing *E. cloacae* and *P. aeruginosa*, in which this latter was not recognized by APS.

EA and CA were obtained in 92.1% of the results. For the Gram-positive organisms, EA and CA were both equal to 96.7% (100% in all cases except 66.7% for erythromycin). For the Gram-negative organisms, EA and CA were both equal to 91.2%, ranging from 70.4% (ceftazidime) to 100% for EA and from 77.8% (ceftazidime) to 100% for CA.

For the 9 cases for which an AST result was not achieved, the APS stopped the run at the end of ID in 4 cases (2 *Streptococcus* spp., a known-off AST panel target, and 2 mixed infection *S. aureus* + *P. aeruginosa*) whereas in 5 cases, containing only one microorganism (1 *K.* oxytoca, 3 *S. aureus*, and 1 CoNS), the run was completed without providing the AST results due to an insufficient control growth. In addition, in 2 cases containing *E. faecalis* only the vancomycin AST failed.

3.3 Time to results

The APS ID results were obtained in 90 min (*versus* 35 min by *Quick*FISH, 90 min by MALDI-TOF directly from BCs and 18-24 h by MALDI-TOF from solid culture) and the AST results were available in 7 h, on average 34.1 h before RLM.

4. Discussion

The APS technology, commercially available as a diagnostic assay, represents a rapid (90 min) and reliable technique for the ID of the most frequent bacteria and yeasts responsible for sepsis and, to our knowledge, the fastest method (7 h) to obtain phenotypic AST directly from a positive BC through determination of MIC.

Althogh the molecular method routinely used (*Quick*FISH) directly from BC is able to achieve an ID result earlier than the APS (35 min *vs* 90 min), it is able to identify only few targets (10 *vs* 16) even if for some of them the APS cannot distinguish among the different species. If the application of MALDI-TOF directly on *Quick*FISH-negative BC can extend the ID to ~~the~~ nearly all the bacteria and yeasts, on the other hand, it requires extra time to complete the ID (90 min besides *Quick*FISH-result) due to~~of~~ technical work (for blood purification and protein extraction) that need several manual steps ~~and for completion (90 min)~~.

In the present study, although on a small number of samples (61), the ID results of the APS were compared with those obtained by RLM and the data in term of specificity (99.6%) were very similar to those (99.5%) reported in a multicenter evaluation performed on 2500 samples (part of which was used to support *in vitro* diagnostic, IVD, classification) (Pancholi et al. 2018) and in a prospective study testing 232 samples (Charnot-Katsikas et al. 2018) and to those (98.9%) reported in a study testing 298 samples using a pre-FDA-cleared software version (Lutgring et al. 2018). In our hands, APS showed ID errors mainly involving *P. aeruginosa* (3 cases: 2 false positives and 1 false negative) and staphylococci (2 cases: 1 *S. aureus* misidentified by APS as CoNS and 1 *S. haemolyticus* not recognized in a sample also containing *E. faecium*), as also reported by Charnot-Katsikas et al. (Charnot-Katsikas et al. 2018). The last error involved a false-negative *S. pneumoniae*, for which *Streptococcus* spp. was the expected APS ID result. In this regard, it would be preferable that APS could achieve differentiation among streptococci at least for the most critical species, such as *S. pneumoniae* and *S. agalactiae*, instead of a genus-level ID, which should be further investigated using alternative methods.

The misidentification of *S. aureus* as CoNS, considered as a VME, can lead to the improper patient management until the ID confirmation by MALDI-TOF from the bacterial growth on solid culture.

Due to the availability of only two modules and to the selection criteria chosen for APS evaluation, the microorganism recovery frequency of this study, if excluding CoNS and *P. aeruginosa*, reflects

that observed in our area in 2018, accounting for 35% of CoNS, 14.5% of *E. coli*, 10.2% of *S. aureus*, 8.6% of *Klebsiella* spp., 6.5% of *Enterococcus* spp., 4% of *Streptococcus* spp., 3% of *P. aeruginosa*, 2.6% of *E. cloacae*, 6.4% of other Gram negative bacteria (mainly including *Acinetobacter* spp. and *S. marcescens*), 5.8 % of other Gram positive bacteria, and 3.4% of yeasts.

Regarding ~~to~~ AST, in our hands the APS performance was encouraging by the absence of VME although the overall EA and CA percentages were lower (92.1% both) than those reported by other Authors (95.1% and 95.5%, respectively, by Charnot-Katsikas et al. and 96.3% and 95.8%, respectively, by Pancholi et al.), as well as the CA (94.1%) reported by Lutgring et al. (Charnot-Katsikas et al. 2018; Lutgring et al. 2018; Pancholi et al. 2018). ~~Our~~ The difference in our results in comparison to those obtained in these studies could be due to ~~different~~ several reasons: i) different comparator methods were used (Vitek 2 and BD Phoenix systems *vs* Vitek 2 for Charnot-Katsikas et al., broth microdilution for Pancholi et al., and MicroScan Walk Away-96 plus for Lutgring et al.); ii) unlike other studies, all AST results obtained by APS were included in the analysis in order to evaluate its performance as a potential sta~~i~~nd-alone diagnostic tool (besides mandatory Gram-staining) and no exclusion criteria were applied; iii) low percentages were observed mainly for Gram-negative (both 91.2%) rather than for Gram-positive (both 96.7%), in agreement with the trend observed in the same studies (EA range 94.4%-95.4% and CA range 93.3%-96.4% for Gram-negative; EA range 97%-97.6% and CA range 97.1%-98.7% for Gram-positive).

In particular, if excluding cefazolin, tested in the APS system only for *Enterobacteriaceae* and for which no comparison was performed due to the lack of the drug in the RLM used, for the Gram-negatives there were 3.6% MEs and 5.1% MiEs among different drugs (6 with ceftazidime, 5 with cefepime, meropenem and piperacillin-tazobactam each, 3 with colistin, 2 with aztreonam and 1 with ciprofloxacin, amikacin, and gentamicin) mainly involving *P. aeruginosa*.

Among Gram-positive cocci, only 2 errors were observed both with erythromycin on *S. aureus*. One of these errors was a ME attributed by APS to a *S. aureus* (also incorrectly classified as MRSA) likely due to the detection of an erythromycin resistance, as revealed by RLM, referred to the *S. salivarius* and *S. mitis*, both contained in the same sample and identified by APS as *Streptococcus* spp., which as expected are ineligible for AST. It is noteworty that APS did not always stop the AST run when more than one microrganism was revealed; this could be misleading since in a routine diagnostic algorithm those results could be the combination of the susceptibility of different microrganisms, producing errors in the administration of a targeted therapy.

Unfortunately, besides the 2 indeterminate results, APS interrupted 6 tests after 90 min, without providing any ID result, likely due to a mute power failure (no apparent malfunction of the system or error message was signaled) which prevented the connection between the two PCs (Control PC and

Analysis PC) of the system leading to the failure of the runs. Moreover in 5 of the 46 AST eligible samples, the run was completed (after about 6 h) without providing the AST results due to an insufficient control growth.

5. Conclusion

In conclusion, the APS system is an automated easy-to-use method requiring only few manual steps and a minimum hands-on time and is potentially able to identify most of the organisms commonly found in BCs, as inferred from our laboratory experience (90.4% of bacteria and 62.1% of yeasts recovered from BCs in 2018 were APS targets, data not shown) and, as already reported by other Authors (Charnot-Katsikas et al. 2018; Pancholi et al. 2018). ~~requires a minimum hands-on-time, and is easy to use~~. However, it suffers from some limitations, such as the number of processable samples per module (only one sample/8 h run/module), the time within which the sample~~s~~ needs to be processed from positive signaling (within 8 h), making APS unsuitable for large-scale sample analysis.~~,~~ ~~and~~ Moreover, it cannot be overlooked the unexpectedly high percentage (18%) of the lack of ID or AST results due to APS technical failures ~~APS ID or AST failure~~ ~~only~~ ~~lately~~ that were displayed only at the end of the ID or AST analysis.

Nonetheless, one of the main advantage of the APS technology is represented by the reduction in the time-to-result of the phenotypic AST, reducing the time to ~~optimize a targeted therapy and allowing to improve clinical outcomes~~ provide the microbiological report to the physician. However, the inability of APS to perform AST for some microorganisms, such as *Streptococcus* spp. and yeasts, could address the use for those microorganisms for which the AST would be performed.

For these reasons, the APS can be potentially used only on a few selected BCs from critical patients, such as those admitted to intensive care unit, as a stand-alone rapid test besides mandatory Gram-staining and subsequent solid culture for ID confirmation and conventional AST completion.

Appendices

Table A.1: Antimicrobial susceptibility testing results provided by Accelerate PhenoTM System (APS) for the 5 Gram-negative challenged strains.

Table A.2: Antimicrobial susceptibility testing results provided by Accelerate PhenoTM System (APS) for the 5 Gram-positive challenged strains.

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Abbreviations

APS: Accelerate Pheno™ System

ID: identification

AST: antimicrobial susceptibility testing

BC: blood culture

PCR: polymerase chain reaction

MALDI-TOF: matrix-assisted laser desorption/ionization-time of flight

PNA-FISH: peptide nucleic acid fluorescent in situ hybridization

GNR: Gram-negative rods

GPC: Gram-positive cocci

RLM: routine laboratory methods

MIC: minimal inhibitory concentration

TP: true positive

FN: false negative

TN: true negative

FP: false positive

EA: essential agreement

CA: categorical agreement

VME: very major error

ME: major error

MiE: minor error

MRSA: methicillin-resistant *S. aureus*

IVD: *in vitro* diagnostic

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Table 1. Results obtained on the challenging samples.

Strain code	Microorganism content	APS ID result
EFM3, EFM7	*Enterococcus faecium*	*Enterococcus faecium*
EFS1	*Enterococcus faecalis*	*Enterococcus faecalis*
SAU3, SAU4	*Staphylococcus aureus*	*Staphylococcus aureus*
ECO1, ECO5	*Escherichia coli*	*Escherichia coli*
KLE1	*Klebsiella pneumoniae*	*Klebsiella* spp.
ENT1	*Enterobacter cloacae*	*Enterobacter* spp.
PAE1	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*

APS: Accelerate Pheno™ System; ID: identification.

Table 2. Identification (ID) results obtained by Accelerate Pheno™ System (APS) compared to routine laboratory methods for the 61 blood culture samples.

	ID results by APS														
	AB	EC	ENT	KLE	PA	SM	CoNS	EFM	EFS	SA	STR	PA+SA	SA+STR	NEG	NO ID
Gram-negative bacteria (34)															
Acinetobacter baumannii (1)	1														
Acinetobacter radioresistens (1)														1	
Bacteroides fragilis (1)														1	
Escherichia coli (15)		13													2
Enterobacter cloacae (1)			1												
Klebsiella oxytoca (3)				3											
Klebsiella pneumoniae (3)				3											
Pseudomonas aeruginosa (7)					6										1
Serratia marcescens (1)						1									
E. cloacae + *P. aeruginosa* (1)			1												
Gram-positive cocci (26)															
Staphylococcus aureus (9)							1			5		2			1
Staphylococcus epidermidis (3)							2								1
Staphylococcus haemolyticus (2)							1								1
Staphylococcus hominis (1)							1								
Enterococcus faecalis (4)									3						1
Enterococcus faecium (2)								2							
Streptococcus pneumoniae (1)														1	
Streptococcus mitis (1)											1				
Streptococcus equi (1)											1				
E. faecium + *S. haemolyticus (1)*								1							
S. aureus + *Streptococcus salivarius* + *S. mitis* (1)													1		
Yeasts (1)															
Candida parapsilosis (1)															1
Total (61)	**1**	**13**	**2**	**6**	**6**	**1**	**5**	**3**	**3**	**5**	**2**	**2**	**1**	**3**	**8**

AB: *Acinetobacter baumannii*; EC: *Escherichia coli*; ENT: *Enterobacter* spp.; KLE: *Klebsiella* spp.; PA: *Pseudomonas aeruginosa*; SM: *Serratia marcescens*; CoNS: coagulase-negative staphylococci; EFM: *Enterococcus faecium*; EFS: *Enterococcus faecalis*; SA: *Staphylococcus aureus*; STR: *Streptococcus* spp.; NEG: negative.

Table 3. Performance of the Accelerate Pheno™ System (APS) obtained on clinical specimens.

APS identification	No. of samples with the following result:				Sensitivity			Specificity		
	TP	FP	TN	FN	No. of TP/(TP+FN)	%	[95% CI]	No. of TN/(TN+FP)	%	[95% CI]
Gram-negative bacteria										
Acinetobacter baumannii	1	0	52	0	1/1	100	[100-100]	52/52	100	[100-100]
Citrobacter spp.	0	0	53	0	0/0	NA	NA	53/53	100	[100-100]
Enterobacter spp.	2	0	51	0	2/2	100	[100-100]	51/51	100	[100-100]
Escherichia coli	13	0	40	0	13/13	100	[100-100]	40/40	100	[100-100]
Klebsiella spp.	6	0	47	0	6/6	100	[100-100]	47/47	100	[100-100]
Proteus spp.	0	0	53	0	0/0	NA	NA	53/53	100	[100-100]
Pseudomonas aeruginosa	6	2	44	1	6/7	85.7	[59.8-100]	44/46	95.7	[89.8-100]
Serratia marcescens	1	0	52	0	1/1	100	[100-100]	52/52	100	[100-100]
Gram-positive cocci										
CoNS	4	1	47	1	4/5	80	[44.9-100]	47/48	97.9	[93.9-100]
Enterococcus faecalis	3	0	50	0	3/3	100	[100-100]	50/50	100	[100-100]
Enterococcus faecium	3	0	50	0	3/3	100	[100-100]	50/50	100	[100-100]
Staphylococcus aureus	8	0	44	1	8/9	88,9	[68.4-100]	44/44	100	[100-100]
Staphylococcus lugdunensis	0	0	53	0	0/0	NA	NA	53/53	100	[100-100]
Streptococcus agalactiae	0	0	53	0	0/0	NA	NA	53/53	100	[100-100]
Streptococcus spp.	3	0	49	1	3/4	75	[32.6-100]	49/49	100	[100-100]
Yeasts										
Candida albicans	0	0	53	0	0/0	NA	NA	53/53	100	[100-100]
Candida glabrata	0	0	53	0	0/0	NA	NA	53/53	100	[100-100]
Total Gram-negative bacteria	29	2	392	1	29/30	96.7	[90.2-100]	392/394	99.5	[98.8-100]
Total Gram-positive cocci	21	1	346	3	21/24	87.5	[74.3-100]	346/347	99.7	[99.1-100]
Total Yeasts	0	0	106	0	0/0	NA	NA	106/106	100	[100-100]
Total	50	3	844	4	50/54	92.6	[85.6-99.6]	844/847	99.6	[99.2-100]

TP: true positive, FP: false positive; TN: true negative; FN: false negative; CI: confidence interval; CoNS: coagulase-negative staphylococci; NA: not applicable.

Table 4. Performance characteristics after discrepancy analysis of the Accelerate Pheno™ System compared to routine laboratory methods for Gram-negative and Gram-positive antimicrobial susceptibility testing by antibiotic and organism grouping.

Antibiotic	No. of combinations	EA			CA			VME (No.)	ME (No.)	MiE (No.)
		No.	%	[95% CI]	No.	%	[95% CI]			
Gram-negative bacteria (n=28)	**330**	**301**	**91.2**	**[88.2-94.3]**	**301**	**91.2**	**[88.2-94.3]**	**0**	**12**	**17**
Amikacin	28	27	96.4	[89.6-100]	27	96.4	[89.6-100]	0	0	1
Ampicillin-sulbactam	19	19	100	[100-100]	19	100	[100-100]	0	0	0
Aztreonam	25	24	96	[88.3-100]	23	92	[81.4-100]	0	0	2
Cefepime	28	22	78.6	[63.4-93.8]	23	82.1	[68-96.3]	0	2	3
Ceftazidime	27	19	70.4	[53.1-87.6]	21	77.8	[62.1-93.5]	0	4	2
Ceftriaxone	21	21	100	[100-100]	21	100	[100-100]	0	0	0
Ciprofloxacin	28	28	100	[100-100]	27	96.4	[89.6-100]	0	0	1
Colistin	26	23	88.5	[76.2-100]	23	88.5	[76.2-100]	0	3	0
Ertapenem	21	21	100	[100-100]	21	100	[100-100]	0	0	0
Gentamicin	27	27	100	[100-100]	26	96.3	[89.2-100]	0	0	1
Meropenem	28	24	85.7	[72.8-98.7]	23	82.1	[68-96.3]	0	1	4
Piperacillin-tazobactam	27	21	77.8	[62.1-93.5]	22	81.5	[66.8-96.1]	0	2	3
Tobramycin	25	25	100	[100-100]	25	100	[100-100]	0	0	0
Gram-positive cocci (n=13)	**60**	**58**	**96.7**	**[92.1-100]**	**58**	**96.7**	**[92.1-100]**	**0**	**1**	**1**
Ampicillin	6	6	100	[100-100]	6	100	[100-100]	0	0	0
Ceftaroline	2	2	100	[100-100]	2	100	[100-100]	0	0	0
Daptomycin	13	13	100	[100-100]	13	100	[100-100]	0	0	0
Doxycycline	6	6	100	[100-100]	6	100	[100-100]	0	0	0
Erythromycin	6	4	66.7	[28.9-100]	4	66.7	[28.9-100]	0	1	1
Linezolid	12	12	100	[100-100]	12	100	[100-100]	0	0	0
Trimethoprim-sulfamethoxazole	4	4	100	[100-100]	4	100	[100-100]	0	0	0
Vancomycin	11	11	100	[100-100]	11	100	[100-100]	0	0	0
Total	**390**	**359**	**92.1**	**[89.4-94.7]**	**359**	**92.1**	**[89.4-94.7]**	**0**	**13**	**18**

EA: essential agreement; CI: confidence interval; CA: category agreement; VME: very major error; ME: major error; MiE: minor error